

## Genetic relationships among the different phenotypes of *Streptococcus dysgalactiae* strains

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The species *Streptococcus dysgalactiae* was proposed to accommodate a heterogeneous group of streptococci associated with infections in animals and human beings. This taxon is now considered to include animal isolates of  $\alpha$ -haemolytic group C streptococci, previously called *S. dysgalactiae*; animal and human isolates of  $\beta$ -haemolytic group C streptococci, previously called '*S. equisimilis*';  $\beta$ -haemolytic group L strains associated with infections in animals and, rarely, in humans; and  $\beta$ -haemolytic group G strains isolated from humans. DNA–DNA reassociation experiments (hydroxyapatite method) and multilocus enzyme electrophoresis (MEE) were performed on reference strains and clinical isolates to determine the genetic relationships among these different phenotypic categories. DNA–DNA hybridization tests showed that they were related at the species level, despite the phenotypic and host heterogeneity. Both genotypic and phenotypic characterization indicated that *S. dysgalactiae* could be separated into two major sub-groups. The first sub-group contained  $\alpha$ -haemolytic strains that showed levels of DNA relatedness with the type strain of *S. dysgalactiae* ranging from 84 to 90% and from 82 to 88% under optimal (55 °C) and stringent (70 °C) conditions, respectively. The second sub-group contained  $\beta$ -haemolytic strains showing levels of relatedness ranging from 71 to 79% (55 °C) and from 62 to 73% (70 °C). Percentage divergence varied from 0.5 to 1.0% ( $\alpha$ -haemolytic group) and from 2.0 to 3.5% ( $\beta$ -haemolytic group). A dendrogram based on phenotypic similarity between the enzyme bands produced by MEE showed a Jaccard similarity coefficient of 0.45 between the subclusters formed by the two sub-groups. The results of phenotypic and genotypic characterization were consistent with a published proposal to divide *S. dysgalactiae* into two subspecies, *S. dysgalactiae* subsp. *dysgalactiae* and *S. dysgalactiae* subsp. *equisimilis*, with a few modifications.

**Keywords:** *Streptococcus dysgalactiae*, multilocus enzyme electrophoresis, DNA–DNA hybridization, genetic relationships

### INTRODUCTION

The classic Lancefield serological grouping has been of major importance in the classification and identification of  $\beta$ -haemolytic streptococci (Lancefield,

1933; Lancefield & Hare, 1935). However, in most cases, when DNA–DNA relatedness studies were applied to the  $\beta$ -haemolytic streptococci, no correlation between genetic relationships and streptococcal group antigens was observed (Kilpper-Bälz & Schleifer, 1984).

Streptococci belonging to Lancefield groups C and G are phenotypically and genotypically diverse. These streptococci can be initially characterized as forming either large or small colonies, which have been differen-

**Abbreviations:** ET, electrophoretic type; MEE, multilocus enzyme electrophoresis; UPGMA, unweighted pair group method with arithmetic averages.

Table 1. Strains used in this study and their sources

Name	Number*	Other designation(s)	Received from†	Source	ET
<i>S. pyogenes</i>	SS 745	ATCC 12344 <sup>‡</sup>	ATCC	Human/group A	1
<i>S. pyogenes</i>	SSI391	A6883	Lämmli	Orangutan/group A	1
<i>S. pyogenes</i>	145-93		CCHSL-UFRJ	Human/group A	2
<i>S. canis</i>	SSI353	ATCC 43396 <sup>‡</sup>	Devriese	Bovine/group G	3
<i>S. canis</i>	CA 146		CCHSL-UFRJ	Canine/group G	4
<i>S. canis</i>	CA 086		CCHSL-UFRJ	Canine/group G	4
<i>S. canis</i>	CA 180		CCHSL-UFRJ	Canine/group G	5
<i>S. canis</i>	SSI389	G2643	Lämmli	Canine/group G	5
<i>S. canis</i>	SSI390	G3232	Lämmli	Canine/group G	5
<i>S. dysgalactiae</i>	SSI346	ATCC 43078 <sup>‡</sup>	ATCC	Bovine/x-haemol. group C	6
<i>S. dysgalactiae</i>	P10		PESAGRO	Bovine/x-haemol. group C	7
<i>S. dysgalactiae</i>	P16		PESAGRO	Bovine/x-haemol. group C	7
<i>S. dysgalactiae</i>	P66		PESAGRO	Bovine/x-haemol. group C	7
<i>S. dysgalactiae</i>	P74		PESAGRO	Bovine/x-haemol. group C	7
<i>S. dysgalactiae</i>	P77		PESAGRO	Bovine/x-haemol. group C	7
<i>S. dysgalactiae</i>	P48		PESAGRO	Bovine/a-haemol. group C	8
<i>S. dysgalactiae</i>	P41		PESAGRO	Bovine/a-haemol. group C	9
<i>S. dysgalactiae</i>	P53		PESAGRO	Bovine/a-haemol. group C	9
<i>S. dysgalactiae</i>	P70		PESAGRO	Bovine/a-haemol. group C	9
<i>S. dysgalactiae</i>	P87		PESAGRO	Bovine/a-haemol. group C	9
<i>S. dysgalactiae</i>	P141		PESAGRO	Bovine/x-haemol. group C	10
<i>Streptococcus</i> sp.	SS18	ATCC 9933	ATCC	Canine/group L	11
<i>Streptococcus</i> sp.	1929-80		CDC	Human vagina/group L	11
<i>Streptococcus</i> sp.	SS87	ATCC 9932	ATCC	Canine/group L	12
<i>Streptococcus</i> sp.	2764-90		CDC	Human/group L	12
<i>Streptococcus</i> sp.	SSI383	L57/6	Lämmli	Bovine/group L	12
<i>Streptococcus</i> sp.	SSI384	T33	Lämmli	Bovine/group L	12
<i>Streptococcus</i> sp.	SSI385	T74	Lämmli	Bovine/group L	12
<i>Streptococcus</i> sp.	SSI386	P473	Lämmli	Porcine/group L	12
<i>Streptococcus</i> sp.	SSI387	957	Lämmli	Porcine/group L	12
<i>Streptococcus</i> sp.	SSI388	1110	Lämmli	Porcine/group L	12
' <i>S. equisimilis</i> '	609-96		CDC	Frog/group C	13
' <i>S. equisimilis</i> '	610-96		CDC	Frog/group C	13
<i>Streptococcus</i> sp.	SSI380	L21454	Lämmli	Bovine/group L	14
<i>Streptococcus</i> sp.	SSI381	L519	Lämmli	Porcine/group L	15
<i>Streptococcus</i> sp.	SSI382	L50/15	Lämmli	Porcine/group L	16
<i>Streptococcus</i> sp.	1375-78		CDC	Food/group L	17
<i>Streptococcus</i> sp.	1376-78		CDC	Food/group L	18
<i>Streptococcus</i> sp.	1609-91		CDC	Human blood/group L	19
' <i>S. equisimilis</i> '	89043		CCHSL-UFRJ	Human/group C	20
' <i>S. equisimilis</i> '	89093		CCHSL-UFRJ	Human/group C	21
<i>Streptococcus</i> sp.	024-91		CCHSL-UFRJ	Human/group G	22
<i>Streptococcus</i> sp.	020-93		CCHSL-UFRJ	Human/group G	23
<i>Streptococcus</i> sp.	009-91		CCHSL-UFRJ	Human/group G	24
<i>Streptococcus</i> sp.	067-93		CCHSL-UFRJ	Human/group G	24
<i>Streptococcus</i> sp.	011-91		CCHSL-UFRJ	Human/group G	24
' <i>S. equisimilis</i> '	022-93		CCHSL-UFRJ	Human/group C	25
' <i>S. equisimilis</i> '	89632		CCHSL-UFRJ	Human/group C	25
' <i>S. equisimilis</i> '	218-84		CDC	Human blood/group C	25
' <i>S. equisimilis</i> '	SS1069		CDC	Human throat/group C	25
' <i>S. equisimilis</i> '	88212		CCHSL-UFRJ	Human/group C	25
' <i>S. equisimilis</i> '	87194		CCHSL-UFRJ	Human/group C	25
' <i>S. equisimilis</i> '	6560-91		CCHSL-UFRJ	Human/group C	26
' <i>S. equisimilis</i> '	SS660	ATCC 35666	ATCC	Human vagina/group C	26

Table 1. (Cont.)

Name	Number*	Other designation(s)	Received from†	Source	ET
'S. equisimilis'	90255		CCHSL-UFRJ	Human/group C	27
'S. equisimilis'	88492		CCHSL-UFRJ	Human/group C	28
'S. equisimilis'	2048-82		CDC	Human/group C	29
Streptococcus sp.	006-91		CCHSL-UFRJ	Human/group G	29
'S. equisimilis'	SS188		CDC	Human throat/group C	30
'S. equisimilis'	75302		CCHSL-UFRJ	Human/group C	31
Streptococcus sp.	80455		CCHSL-UFRJ	Human/group G	31
'S. equisimilis'	115-84		CDC	Human blood/group C	32
Streptococcus sp.	184-93		CCHSL-UFRJ	Human/group G	33
'S. equisimilis'	001-94		CCHSL-UFRJ	Human/group C	33
'S. equisimilis'	88296		CCHSL-UFRJ	Human/group C	34
Streptococcus sp.	049-93		CCHSL-UFRJ	Human/group G	35
Streptococcus sp.	81577		CCHSL-UFRJ	Human/group G	36
'S. equisimilis'	3296-94		CDC	Human/group C	36
Streptococcus sp.	007-91		CCHSL-UFRJ	Human/group G	37
Streptococcus sp.	033-91		CCHSL-UFRJ	Human/group G	38
Streptococcus sp.	183-93		CCHSL-UFRJ	Human/group G	39
Streptococcus sp.	85304		CCHSL-UFRJ	Human/group G	39
Streptococcus sp.	005-91		CCHSL-UFRJ	Human/group G	39
Streptococcus sp.	030-91		CCHSL-UFRJ	Human/group G	40
Streptococcus sp.	034-91		CCHSL-UFRJ	Human/group G	41
Streptococcus sp.	035-91		CCHSL-UFRJ	Human/group G	41
Streptococcus sp.	039-91		CCHSL-UFRJ	Human/group G	42
Streptococcus sp.	120-93		CCHSL-UFRJ	Human/group G	42
'S. equisimilis'	CA125		CCHSL-UFRJ	Canine/group C	43
'S. equisimilis'	1775-93		CDC	Human/group C	43
'S. equisimilis'	1778-93		CDC	Human/group C	44
'S. equisimilis'	91069		CCHSL-UFRJ	Human/group C	45
'S. equisimilis'	1779-93		CDC	Human/group C	46
Streptococcus sp.	044-91		CCHSL-UFRJ	Human/group G	47
'S. equisimilis'	SS957		CDC	Human/group C	48
Streptococcus sp.	025-91		CCHSL-UFRJ	Human/group G	49
Streptococcus sp.	032-91		CCHSL-UFRJ	Human/group G	50
Streptococcus sp.	026-91		CCHSL-UFRJ	Human/group G	50
Streptococcus sp.	037-91		CCHSL-UFRJ	Human/group G	50
'S. equisimilis'	91071		CCHSL-UFRJ	Human/group C	50
Streptococcus sp.	105-93		CCHSL-UFRJ	Human/group G	51
Streptococcus sp.	78064			Human/group G	51
'S. equisimilis'	024-93		CCHSL-UFRJ	Human/group C	52
Streptococcus sp.	019-91		CCHSL-UFRJ	Human/group G	53
Streptococcus sp.	81752		CCHSL-UFRJ	Human/group G	54
Streptococcus sp.	81038		CCHSL-UFRJ	Human/group G	55
'S. equisimilis'	040-93		CCHSL-UFRJ	Human/group C	56
'S. equisimilis'	3110-94		CDC	Human blood/group C	56
Streptococcus sp.	042-91		CCHSL-UFRJ	Human/group G	57
'S. equisimilis'	2466-91		CCHSL-UFRJ	Human/group C	58
'S. equisimilis'	91082		CCHSL-UFRJ	Human abscess/group C	59
Streptococcus sp.	003-91		CCHSL-UFRJ	Human/group G	60
Streptococcus sp.	016-91		CCHSL-UFRJ	Human/group G	61
Streptococcus sp.	010-91		CCHSL-UFRJ	Human/group G	61
Streptococcus sp.	89191		CCHSL-UFRJ	Human/group G	62
Streptococcus sp.	81541		CCHSL-UFRJ	Human/group G	63
Streptococcus sp.	015-91		CCHSL-UFRJ	Human/group G	63
Streptococcus sp.	022-91		CCHSL-UFRJ	Human/group G	64

Table 1. (Cont.)

Name	Number*	Other designation(s)	Received from†	Source	ET
<i>Streptococcus</i> sp.	81794		CCHSL-UFRJ	Human/group G	65
<i>S. equi</i> subsp. <i>equi</i>	SS1344	ATCC 33398 <sup>†</sup>	ATCC	Horse	66
<i>S. equi</i> subsp. <i>zooepidemicus</i>	SS1345	ATCC 43079 <sup>†</sup>	ATCC	Horse	67
<i>S. iniae</i>	2378-91		CDC	Human blood	68
<i>S. iniae</i>	SS1123	ATCC 29177	ATCC	Dolphin	68
<i>S. iniae</i>	SS1056	ATCC 29178 <sup>†</sup>	ATCC	Dolphin	69
<i>S. iniae</i>	SS1327		CDC	Tilapia	69
<i>S. iniae</i>	SS1323		CDC	Tilapia	69
<i>S. iniae</i>	SS1331		CDC	Tilapia	69
<i>S. iniae</i>	SS1392	10 A	CDC	Tilapia	69
<i>S. iniae</i>	SS1393	10 J	CDC	Tilapia	69
<i>S. iniae</i>	SS1394	11 F	CDC	Sea bass	69
<i>S. agalactiae</i>	SS617		CDC	Human	70

\* SS, Standard strain from the Culture Collection of the *Streptococcus* Laboratory, Centers for Disease Control and Prevention, Atlanta, GA, USA.

† ATCC, American Type Culture Collection, Rockville, MD, USA; CCHSL-UFRJ, Culture Collection of the  $\beta$ -Hemolytic Streptococci Laboratory, Federal University of Rio de Janeiro, Brazil; CDC, Centers for Disease Control and Prevention, Atlanta, GA, USA; PESAGRO, Pesquisas Agropecuárias do Rio de Janeiro, Brazil; Devriese, L. A. Devriese, University of Ghent, Belgium; Lämmler, Ch. Lämmler, Justus-Liebig Universität, Germany.

tiated on the basis of biochemical tests (Cimolai & Mah, 1991; Lawrence *et al.*, 1985) and DNA relatedness studies (Ezaki *et al.*, 1986). The distinction between the colony forms is important because they have a different spectrum of clinical manifestations. The small-colony-forming strains belong to the '*Streptococcus milleri*' group. Two major categories of the large-colony-forming Lancefield group C and G streptococci are considered to correspond to different species: the equine  $\beta$ -haemolytic *Streptococcus equi* (group C) with its subspecies *equi* and *zooepidemicus* (Farrow & Collins, 1984), and *Streptococcus canis* (group G), carried by dogs and cats and an infrequent cause of mastitis in cattle (Devriese *et al.*, 1986). Another species name, *Streptococcus dysgalactiae*, was revived by Garvie *et al.* (1983), but was considered to be restricted to  $\alpha$ -haemolytic group C strains of bovine origin. Subsequently, the DNA hybridization studies of Farrow & Collins (1984) demonstrated that *S. dysgalactiae* exhibited high levels of DNA relatedness with large-colony-forming Lancefield group C ('*S. equisimilis*'), human group G and group L  $\beta$ -haemolytic streptococci, suggesting that this group of organisms belong to a single species, *S. dysgalactiae*. These findings were confirmed by the studies of Kilpper-Bälz & Schleifer (1984) and Ezaki *et al.* (1986). The species consists of distinct sub-groups based on serogroups, biotypes and hosts: (i) *S. dysgalactiae*,  $\alpha$ -haemolytic group C streptococci that can cause bovine mastitis; (ii)  $\beta$ -haemolytic group C strains named '*S. equisimilis*' that can cause disease in animals; (iii)  $\beta$ -haemolytic group L strains that have been associated with infections in pigs, dogs, cattle and other animals,

but rarely in humans; (iv)  $\beta$ -haemolytic group C strains, also named '*S. equisimilis*', isolated from humans; and (v)  $\beta$ -haemolytic group G strains isolated from humans. Both group C and G streptococci have been recovered from humans as commensals as well as pathogens (Beebe & Koneman, 1995; Cimolai *et al.*, 1991; Lancefield & Hare, 1935).

The phenotypic diversity found among components of this species has prompted investigations about the relationships among the sub-groups and possible division of the proposed species *S. dysgalactiae*. Elstratiou *et al.* (1994) observed biochemical differences among some sub-groups of this species. Vandamme *et al.* (1996) showed that these bacteria could be allocated into subspecies based on their physiological and biochemical properties and by numerical analysis of whole-cell protein profiles. The name *S. dysgalactiae* subsp. *equisimilis* was proposed for human strains belonging to groups C and G, and strains of animal origin were placed in a distinct subspecies for which the name *S. dysgalactiae* subsp. *dysgalactiae* was proposed (Vandamme *et al.*, 1996).

Methods for measuring genetic relatedness of these organisms have been employed in only a few of these studies. The purpose of the present study was to determine genetic relationships among strains belonging to Lancefield groups C, G and L, with emphasis on those resembling *S. dysgalactiae*, by using multilocus enzyme electrophoresis (MEE) and DNA hybridization.

## METHODS

**Bacterial strains.** The 121 streptococcal strains used in the study and their sources are listed in Table 1.

**Phenotypic characterization of strains.** The strains were characterized on the basis of serological grouping performed by the Lancefield hot-acid extraction procedure and precipitin reactions with specific antisera for group A, B, C, G and L streptococci prepared at the Centers for Disease Control and Prevention, Atlanta, GA, USA, or at the Institute of Microbiology, Federal University of Rio de Janeiro, Brazil. Phenotypic characteristics were tested by conventional biochemical tests as described previously (Facklam & Washington, 1991). Strains were additionally tested using API 20 STREP (bioMérieux) according to the manufacturer's instructions. They were also tested for  $\beta$ -D-glucuronidase,  $\beta$ -D-galactosidase and  $\alpha$ -D-galactosidase activities, by using 4-methylumbelliferyl- $\alpha$ -D-galactoside substrates as described by Sliifkin & Gil (1983) and Whitley *et al.* (1990). Hyaluronidase and lysis of human fibrin were determined according to the method previously described (Vieira & Castro, 1994).

**Preparation of bacterial extracts for MEE.** Each strain was grown in 100 ml Todd-Hewitt broth (THB) at 37 °C and harvested by centrifugation at 1000 g for 12 min at 4 °C. The cells were suspended in 200  $\mu$ l lysis buffer containing 1% (v/v) Triton X-100, 40 mM potassium phosphate buffer (pH 7.5), 1 mM EDTA and 1 mM DTT. To each sample was added 100 mg 0.1 mm diameter glass beads (Sigma), and the cells were homogenized in a vortex stirrer for 3 min. After centrifugation at 12000 g for 30 s, the supernatant (lysate) was stored at -20 °C until being tested by MEE.

**MEE.** The bacterial lysates were subjected to electrophoresis on 1% agarose horizontal gels according to procedures previously described (Momen & Salles, 1985). Optimal electrophoretic conditions for each enzyme were determined by testing various buffer systems and variables as recommended by Selander *et al.* (1986). Thirty-three enzymic loci were screened for activity, but only the following 10 gave a consistent banding patterns for all strains and were used thereafter: nucleoside phosphorylase, EC 2.4.2.1 (NP); leucylalanine peptidase, EC 3.4.11.1 (LAP); NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, EC 1.2.1.12 (GAPD1); NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, EC 1.2.1.13 (GAPD2); leucine aminopeptidase, EC 3.4.11.1 (LAP);  $\beta$ -D-glucuronidase, EC 3.2.1.31 ( $\beta$ -GUS); mannose-6-phosphate isomerase, EC 5.3.1.8 (MPI); leucylproline peptidase, EC 3.4.13.9 (PEPD); phosphoglucose isomerase, EC 5.3.1.9 (GPI); NAD-glycohydrolase, EC 3.2.2.6 (NADG). The electrophoresis buffer for the enzymes NP, PEP2, GAPD1, MPI, PEPD and GPI was Tris/citrate (pH 8.0). A sodium phosphate buffer (pH 8.0) was used for electrophoresis of NADG, and Tris/citrate buffer (pH 6.3) was used for GAPD2, LAP and  $\beta$ -GUS. The staining methods used for NP, PEP2, PEPD and GPI were as described by Zahner *et al.* (1989). The enzyme MPI was stained according to the method of Cupolillo *et al.* (1994) and NADG as described by Harris & Hopkinson (1976). For GAPD1, GAPD2 and  $\beta$ -GUS the staining procedures were performed as recommended by Pasteur *et al.* (1988) and for LAP as described by Selander *et al.* (1986). The distinctive electromorphs (mobility variants) of each enzyme were numbered in order of decreasing rate of anodal migration and were equated with alleles at corresponding structural gene loci. Strains that shared identical allelic profiles at all enzyme loci examined

were recorded as belonging to the same electrophoretic type (ET).

**Analysis of MEE data.** The numerical analysis was performed using the NTSYS-pc software package (F. James Rohlf, version 1.7, Exeter Software). The Jaccard ( $S_j$ ) and the simple-matching-type ( $S_{sm}$ ) similarity coefficients (Sneath & Sokal, 1973) were used to determine the relationships between the ETs. For this analysis, a matrix with presence/absence of bands was built. The similarity matrix was transformed into a dendrogram by the unweighted pair group method with arithmetic averages (UPGMA). Co-phenetic correlation coefficients (CCCs) were determined (Sneath & Sokal, 1973) to assess the agreement between similarity values implied by the phenogram and those of the original similarity matrix. To estimate the genetic distance among species or groups of  $\beta$ -haemolytic streptococci a matrix of allele frequencies (electromorphs) was constructed and the Nei genetic distance between each pair of species was calculated. A phenogram was created from the matrix of Nei distances by the UPGMA clustering algorithm (Sneath & Sokal, 1973).

**DNA-DNA reassociation experiments.** DNA-DNA reassociation studies were performed with selected strains. The strains were grown in 2 l THB at 37 °C for 24 h with gentle shaking. Harvesting and lysis of the bacterial cells were performed as described by Teixeira *et al.* (1994), except that 500  $\mu$ l mutanolysin (2000 U ml<sup>-1</sup>) was also added to weaken the cell walls and the cells were incubated for 2.5 h instead of 30 min. The procedures used to purify DNA and to determine DNA relatedness by the hydroxyapatite method have been described previously (Brenner *et al.*, 1982). DNA was labelled enzymically *in vitro* with [<sup>32</sup>P]dCTP by using a nick translation reagent kit (Gibco-BRL) as directed by the manufacturer. DNA hybridization experiments were performed at 55 °C for optimal DNA reassociation and at 70 °C for stringent conditions. Levels of divergence within related sequences were determined by assuming that each degree of heteroduplex instability, compared with the melting temperature of the homologous duplex, was caused by approximately 1% of unpaired bases. Percentage divergence was calculated to the nearest 0.5%.

## RESULTS

All strains of *S. dysgalactiae* sub-groups were positive for acid production from ribose, starch and trehalose and produced alkaline phosphatase, arginine dehydrolase, leucine arylamidase and  $\beta$ -D-glucuronidase. Most of the strains produced hyaluronidase. Strains of all *S. dysgalactiae* sub-groups were negative for acetoin (Voges-Proskauer) production, and also for acid production from arabinose, inulin and raffinose. Most strains failed to produce pyrrolidonylarylamidase. Fifty-one per cent of the strains were positive for acid production from lactose.

Variable results were obtained for some characteristics when different methods were compared. According to the results of conventional tests, 47% of the strains gave positive reactions for aesculin hydrolysis, whilst only 30% of the strains were positive when the API 20 STREP was used. Similar findings were observed with  $\beta$ -D-galactosidase production. All strains were negative for  $\beta$ -D-galactosidase activity, as determined by API 20 STREP, although most of the  $\beta$ -haemolytic *S. dysga-*

**Table 2.** Phenotypic characteristics useful for the differentiation of *S. dysgalactiae* strains

Sub-group	No. of strains	Phenotypic characteristic*							
		HAEM	SOR	GLY	$\beta$ -D-GAL	HIP	PAHF	BAC	$\alpha$ -L-GLU
<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>	12	$\alpha$	(+)	—	—	—	—	S	—
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>									
Human group C	30	$\beta$	—	—	+	—	+	R	+
Animal group C	3	$\beta$	—	+	+	—	—	R	+
Human group G	39	$\beta$	—	—	+	—	+	R	+
Group L	16	$\beta$	—	+	+	(+)	—	S	+

\* HAEM, haemolysis; SOR, acid from sorbitol; GLY, acid from glycogen;  $\beta$ -D-GAL,  $\beta$ -D-galactosidase; HIP, hydrolysis of hippurate; PAHF, proteolytic activity on human fibrin; BAC, bacitracin;  $\alpha$ -L-GLU,  $\alpha$ -L-glutamate aminopeptidase; +, 95% or more of strains positive; (—), 70–80% of strains positive; —, 95% or more of strains negative; S, susceptible; R, resistant.

*lactiae* sub-groups were positive when determined by using 4-methylumbelliferyl-conjugated  $\beta$ -D-galactoside as substrate. The differential phenotypic characteristics of *S. dysgalactiae* sub-groups are listed in Table 2.

All 10 enzymes assayed were polymorphic among the 121 streptococci strains tested. The mean number of alleles per locus was 5.2. Seventy ETs were identified. The electromorphic profiles of the ETs and the distribution of the strains into ETs are given in Table 3.

The relationships of the ETs are shown in the phenogram obtained using  $S_1$ /UPGMA (Fig. 1). The clusters obtained by  $S_{SM}$ /UPGMA (data not shown) corresponded to the clusters found by  $S_1$ /UPGMA. The  $S_1$ /UPGMA phenogram had high cophenetic correlation ( $r = 0.90$ ). In this phenogram the ETs fell into six major clusters (marked I–VI) at the 0.25  $S_1$  level. The clusters corresponded to different *Streptococcus* species: *S. pyogenes*, *S. canis*, *S. dysgalactiae*, *S. equi*, *S. infantis* and *S. agalactiae*, respectively. Each cluster had distinctive ETs and there was no sharing of ETs among the species studied. If a similarity value of 0.45 is considered, the ETs within cluster III could be divided into three distinctive subclusters. Subcluster a (ET 6–10) contains the  $\alpha$ -haemolytic group C streptococci. Subcluster b (ET 11–16) contains most of the strains of group L streptococci and two animal strains (ET 13) of *S. equisimilis*. This subcluster was characterized by all the strains having allele PEP2 1. Subcluster c (ET 17–65) includes strains of groups C, G and L streptococci. Some group L streptococcal strains (ET 17–19) still remained linked to human groups C and G streptococci because of the close association of ETs. Five ETs (ET 29, 31, 33, 36 and 50) were common to group G streptococci and human group C streptococci (*S. equisimilis*).

Fig. 2 shows the genetic distance values among streptococci. The species were clustered according to the results obtained when the strains were compared by phenetic analysis.

DNA hybridization studies performed by using labelled DNAs from the type strain of *S. dysgalactiae*, ATCC 43078, an  $\alpha$ -haemolytic group C strain or from ATCC 35666, a human  $\beta$ -haemolytic group C strain of *S. equisimilis*, revealed that all *S. dysgalactiae* strains tested were in a single DNA relatedness group (Table 4). Two sub-groups were evident. DNA relatedness sub-group 1 contained all group C  $\alpha$ -haemolytic *S. dysgalactiae* strains tested. The human group G streptococci, *S. equisimilis* and group L streptococci were in DNA relatedness sub-group 2. The levels of DNA relatedness among strains belonging to group 1 and the type strain ATCC 43078 at optimal re-association conditions (55 °C) ranged from 84 to 90% with 0.5–1.0% divergence. They were 82–88% related under stringent DNA reassociation conditions (70 °C). Relatedness of the type strain to strains of sub-group 2 was 71–79% with 2.0–3.5% divergence at 55 °C, and 62–73% at 70 °C. Similar results were obtained in reciprocal experiments using labelled DNA from DNA sub-group 2 strain ATCC 35666. The sub-group 2 strains were 77–90% related with 0.5–2.0% divergence at 55 °C and 71–87% related at 70 °C. Relatedness of sub-group 1 strains to ATCC 35666 was 73–76% with 2.5–3.5% divergence at 55 °C, and 59–69% at 70 °C.

## DISCUSSION

The phenotypic sub-groups of *S. dysgalactiae* are of importance in human and veterinary medicine. Those from veterinary sources have been considered as a cause of animal infections. In recent years, there has been increasing recognition of the pathogenic potential

Table 3. Allelic profiles at 10 enzyme loci for 70 ETs of streptococci

ET	No. of Strains	Group	Species	Allele at enzyme locus*									
				NP	PEP2	GAPD1	GAPD2	LAP	$\beta$ -GUS	MPI	PEPD	GPI	NADG
1	2	A	<i>S. pyogenes</i>	3	1	5	3	5	0	1	5	4	6
2	1	A	<i>S. pyogenes</i>	3	2	5	3	5	0	1	4	4	6
3	1	G	<i>S. canis</i>	2	2	6	3	3	0	4	4	4	3
4	2	G	<i>S. canis</i>	2	2	6	3	5	0	4	4	4	3
5	3	G	<i>S. canis</i>	2	2	6	3	5	0	4	3	4	3
6	1	C	<i>S. dysgalactiae</i>	2	2	3	3	5	5	6	6	4	5
7	5	C	<i>S. dysgalactiae</i>	3	2	3	3	5	5	6	6	4	5
8	1	C	<i>S. dysgalactiae</i>	3	2	3	3	5	4	6	6	4	5
9	4	C	<i>S. dysgalactiae</i>	3	2	5	3	5	5	6	6	4	5
10	1	C	<i>S. dysgalactiae</i>	3	2	5	3	5	4	6	6	4	5
11	2	L	<i>Streptococcus</i> sp.	3	1	4	3	3	4	5	6	4	7
12	8	L	<i>Streptococcus</i> sp.	3	1	4	3	3	4	6	6	4	7
13	2	C	' <i>S. equisimilis</i> '	3	1	4	3	4	4	6	6	4	7
14	1	L	<i>Streptococcus</i> sp.	3	1	3	3	3	4	6	6	4	7
15	1	L	<i>Streptococcus</i> sp.	3	1	3	3	3	3	6	6	4	7
16	1	L	<i>Streptococcus</i> sp.	3	1	4	3	3	3	6	6	4	7
17	1	L	<i>Streptococcus</i> sp.	2	2	4	3	3	4	5	6	4	7
18	1	L	<i>Streptococcus</i> sp.	2	2	4	3	3	4	6	6	4	7
19	1	L	<i>Streptococcus</i> sp.	2	2	4	3	4	4	6	6	4	7
20	1	C	' <i>S. equisimilis</i> '	2	2	2	3	3	3	6	6	4	7
21	1	C	' <i>S. equisimilis</i> '	2	2	2	3	3	4	6	6	4	7
22	1	G	<i>Streptococcus</i> sp.	2	2	2	3	3	2	6	6	4	7
23	1	G	<i>Streptococcus</i> sp.	2	2	1	3	3	3	6	6	4	7
24	3	G	<i>Streptococcus</i> sp.	2	2	1	3	3	4	6	6	4	7
25	6	C	' <i>S. equisimilis</i> '	2	2	2	3	4	2	5	6	4	7
26	2	C	' <i>S. equisimilis</i> '	2	2	1	3	3	1	5	6	4	7
27	1	C	' <i>S. equisimilis</i> '	2	2	2	3	2	5	6	6	4	7
28	1	C	' <i>S. equisimilis</i> '	2	2	2	3	3	1	5	6	4	7
29	2	C, G	<i>Streptococcus</i> sp.	2	2	2	3	3	5	6	6	4	7
30	1	C	' <i>S. equisimilis</i> '	2	2	1	3	3	1	5	6	4	7
31	2	C, G	<i>Streptococcus</i> sp.	2	2	1	3	3	2	5	6	4	7
32	1	C	' <i>S. equisimilis</i> '	3	2	3	3	3	2	5	6	4	7
33	2	C, G	<i>Streptococcus</i> sp.	2	2	3	3	3	2	5	6	4	7
34	1	C	' <i>S. equisimilis</i> '	2	2	4	3	4	3	5	6	4	7
35	1	G	<i>Streptococcus</i> sp.	2	2	4	3	3	3	5	6	4	7
36	2	C, G	<i>Streptococcus</i> sp.	2	2	2	3	3	3	5	6	4	7
37	1	G	<i>Streptococcus</i> sp.	2	2	3	3	4	3	5	6	4	7
38	1	G	<i>Streptococcus</i> sp.	2	1	2	3	3	3	5	6	4	8
39	3	G	<i>Streptococcus</i> sp.	2	2	3	3	3	2	5	6	4	8
40	1	G	<i>Streptococcus</i> sp.	2	2	3	3	3	0	5	6	4	8
41	2	G	<i>Streptococcus</i> sp.	2	2	3	3	3	3	5	6	4	8
42	2	G	<i>Streptococcus</i> sp.	2	2	3	3	3	4	5	6	4	8
43	2	C	' <i>S. equisimilis</i> '	3	2	4	3	3	4	5	6	4	7
44	1	C	' <i>S. equisimilis</i> '	3	2	4	3	2	4	5	6	4	7
45	1	C	' <i>S. equisimilis</i> '	2	2	4	3	2	4	5	6	4	7
46	1	C	' <i>S. equisimilis</i> '	2	2	2	3	2	4	5	6	4	7
47	1	G	<i>Streptococcus</i> sp.	3	2	2	3	2	4	5	6	4	7
48	1	C	' <i>S. equisimilis</i> '	3	2	3	3	4	0	5	6	4	7
49	1	G	<i>Streptococcus</i> sp.	3	2	3	3	4	4	5	6	4	7
50	4	1C, 3G	<i>Streptococcus</i> sp.	3	2	3	3	3	4	5	6	4	7
51	2	G	<i>Streptococcus</i> sp.	3	2	3	3	2	4	5	6	4	7
52	1	G	<i>Streptococcus</i> sp.	3	2	3	3	2	3	5	6	4	7
53	1	G	<i>Streptococcus</i> sp.	3	2	3	3	3	3	5	6	4	7

Table 3. (Cont.)

ET	No. of Strains	Group	Species	Allele at enzyme locus*									
				NP	PEP2	GAPD1	GAPD2	LAP	$\beta$ -GUS	MPI	PEPD	GPI	NADG
54	1	G	<i>Streptococcus</i> sp.	3	2	3	3	3	3	5	6	4	8
55	1	G	<i>Streptococcus</i> sp.	3	2	3	3	4	0	5	6	4	8
56	2	C	' <i>S. equisimilis</i> '	3	2	2	3	3	3	5	6	4	7
57	1	G	<i>Streptococcus</i> sp.	3	2	2	3	3	0	5	6	4	7
58	1	C	' <i>S. equisimilis</i> '	3	2	2	3	3	4	5	6	4	7
59	1	C	' <i>S. equisimilis</i> '	3	2	2	3	3	2	5	6	4	7
60	1	G	<i>Streptococcus</i> sp.	3	2	2	3	3	0	6	6	4	7
61	2	G	<i>Streptococcus</i> sp.	3	2	2	3	3	4	6	6	4	7
62	1	G	<i>Streptococcus</i> sp.	3	2	2	3	3	2	6	6	4	7
63	2	G	<i>Streptococcus</i> sp.	3	2	2	3	3	3	6	6	4	7
64	1	G	<i>Streptococcus</i> sp.	3	2	2	3	4	4	6	6	4	7
65	1	G	<i>Streptococcus</i> sp.	3	2	2	3	4	3	6	6	4	7
66	1	C	<i>S. equi</i> subsp. <i>equi</i>	2	1	6	1	1	1	7	3	2	2
67	1	C	<i>S. equi</i> subsp. <i>zooepidemicus</i>	1	1	6	2	1	1	8	3	2	2
68	2	-	<i>S. iniae</i>	3	1	5	2	2	1	3	1	1	4
69	7	-	<i>S. iniae</i>	2	1	5	2	2	1	3	1	1	4
70	1	B	<i>S. agalactiae</i>	1	3	6	4	0	1	2	2	3	1

\* NP, Nucleoside phosphorylase; PEP2, leucylalanine peptidase; GAPD1, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase; GAPD2, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase; LAP, leucine aminopeptidase;  $\beta$ -GUS,  $\beta$ -D-glucuronidase; MPI, mannose-6-phosphate isomerase; PEPD, leucylproline peptidase; GPI, phosphoglucose isomerase; NADG, NAD-glycohydrolase.

of group G streptococci and '*S. equisimilis*', sub-groups of *S. dysgalactiae* isolated from humans. These organisms have a similar clinical spectrum. They can cause a variety of illnesses, ranging from acute pharyngitis (Cimolai *et al.*, 1991) to severe infections, such as septicæmia and endocarditis (Bucher & Gaustad, 1990; Carmeli *et al.*, 1995). Many of these infections have occurred in patients with some degree of immunosuppression. Recently, Beebe & Koneman (1995) described a possible association of group G with neoplastic disease. Consequently, the establishment of genetic relationships among *S. dysgalactiae* sub-groups is necessary for answering certain basic questions concerning the epidemiology, pathogenicity and classification of these organisms.

MEE has been successfully applied to taxonomic studies of many organisms (Balmelli & Piffaretti, 1996; Cupolillo *et al.*, 1994; Musser *et al.*, 1986). Although this method has not been used to classify strains to the subspecies level, MEE results were compatible with the results of DNA-DNA hybridization, a method considered the standard reference technique in bacterial species identification. Subspecific classification may also reflect differences in DNA relatedness, in addition to other criteria such as phenotypic characters, pathogenicity and host range (Wayne *et al.*, 1987). We used MEE to determine the phenetic and genetic affinities among the sub-groups of *S. dysgalactiae*. In this study two different techniques were used in the analysis of

the MEE data. The first analysis used a coefficient of similarity to determine the phenetic relationships between ETs (Fig. 1). In the second analysis, genotypes were inferred from electromorphs, and from these data a measure of genetic distance among the sub-groups was calculated (Fig. 2). Similar classifications were obtained using the two different analyses. The results showed that the species of pyogenic streptococci could be differentiated by this method. MEE data could also be used to distinguish between  $\alpha$ -haemolytic and  $\beta$ -haemolytic *S. dysgalactiae* strains. Our data confirm a previous report of Bert *et al.* (1995), which showed that MEE had potential for the differentiation of pyogenic streptococci. These authors did not report separation of the sub-groups of this species. We used different enzymic systems, buffer systems and gels than those used by Bert *et al.* (1995) to achieve differentiation of the subspecies of *S. dysgalactiae*.

DNA relatedness has been recommended as the method of choice for differentiating species (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). The phylogenetic definition of a species is a group of 'strains with approximately 70% or greater DNA-DNA relatedness and with 5 °C or less  $\Delta T_m$ '. Both values must be considered (Wayne *et al.*, 1987). It has also been demonstrated that the subspecies concept is phylogenetically valid, on the basis of the frequency of distribution of levels of divergence within related sequences and preferentially high-level of intraspecies



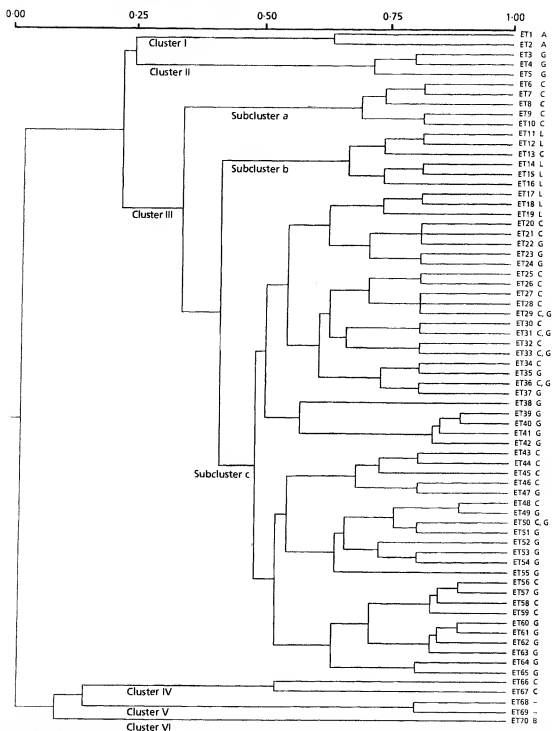


Fig. 1. Phenogram showing the Jaccard similarity coefficient between the ETs of streptococcal strains. Clusters I, II, III, IV, V and VI correspond to *S. pyogenes*, *S. canis*, *S. dysgalactiae*, *S. equi*, *S. iniae* and *S. agalactiae*, respectively. Subcluster a contains  $\alpha$ -haemolytic *S. dysgalactiae* strains; subcluster b contains  $\beta$ -haemolytic group L streptococci and animal group C (*S. equisimilis*), and subcluster c contains  $\beta$ -haemolytic groups C, G and L streptococci.

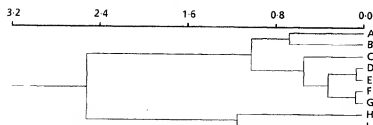


Fig. 2. Nei genetic distance between streptococcal species and sub-groups of *S. dysgalactiae*: A, *S. pyogenes*; B, *S. canis*; C,  $\alpha$ -haemolytic *S. dysgalactiae* strains; D, human  $\beta$ -haemolytic group C streptococci; E, human  $\beta$ -haemolytic group G streptococci; F, human  $\beta$ -haemolytic group L streptococci; G, animal  $\beta$ -haemolytic group C streptococci; H, *S. equi*; I, *S. iniae*.

Table 4. Levels of DNA relatedness of *S. dysgalactiae* and other *Streptococcus* species

All reactions were done in duplicate.

Source of unlabelled DNA	Relative binding (%) with labelled DNA from:			
	<i>S. dysgalactiae</i> ATCC 43078 <sup>T</sup>		<i>S. equisimilis</i> ATCC 35666	
	55 °C (% D*)	70 °C	55 °C (% D*)	70 °C
<i>S. dysgalactiae</i> ATCC 43078 <sup>T</sup>	100 (0.0)	100	75 (2.5)	64
<i>S. dysgalactiae</i> P 41	90 (1.0)	88	73 (3.0)	69
<i>S. dysgalactiae</i> P 48	88 (1.0)	86	76 (3.0)	67
<i>S. dysgalactiae</i> P 70	89 (1.0)	85	74 (3.5)	59
<i>S. dysgalactiae</i> P 72	84 (0.5)	87	74 (3.5)	61
<i>S. dysgalactiae</i> P 74	90 (0.5)	87	74 (3.5)	69
<i>S. dysgalactiae</i> P 87	86 (0.5)	82	76 (3.5)	66
<i>S. equisimilis</i> ATCC 35666	77 (2.5)	68	100 (0.0)	100
<i>S. equisimilis</i> SS1069	77 (2.5)	69	88 (1.0)	81
<i>S. equisimilis</i> SS188	75 (2.5)	69	88 (0.5)	86
<i>S. equisimilis</i> 2466-91	71 (2.5)	65	83 (1.0)	81
<i>S. equisimilis</i> 88296	71 (2.5)	64	84 (1.0)	87
<i>S. equisimilis</i> CDC 115-84	76 (3.0)	65	83 (1.0)	83
<i>S. equisimilis</i> CDC 3296-94	71 (2.5)	62	89 (0.5)	85
<i>S. equisimilis</i> 25	76 (3.0)	72	83 (1.0)	80
<i>S. equisimilis</i> CDC 609-96	77 (2.5)	73	83 (1.0)	82
<i>S. equisimilis</i> CDC 610-96	76 (2.5)	71	84 (1.0)	81
Group G <i>Streptococcus</i> ATCC 12394	79 (3.0)	72	85 (1.0)	80
Group G <i>Streptococcus</i> 049-93	76 (3.5)	68	84 (1.0)	81
Group G <i>Streptococcus</i> 120-93	77 (3.0)	69	92 (1.0)	84
Group G <i>Streptococcus</i> 006-91	75 (3.5)	69	88 (1.0)	85
Group G <i>Streptococcus</i> 032-91	76 (3.0)	67	88 (1.5)	82
Group G <i>Streptococcus</i> 042-91	77 (3.0)	71	89 (1.5)	84
Group L <i>Streptococcus</i> ATCC 9933	77 (2.0)	68	78 (2.0)	71
Group L <i>Streptococcus</i> ATCC 9932	79 (2.0)	70	81 (2.0)	74
Group L <i>Streptococcus</i> CDC 1375-91	72 (2.0)	68	76 (1.5)	73
Group L <i>Streptococcus</i> CDC 1376-91	74 (2.0)	69	77 (2.0)	73
Group L <i>Streptococcus</i> CDC 1929-80	78 (2.0)	70	77 (1.5)	75
Group L <i>Streptococcus</i> CDC 2764-90	77 (3.0)	69	77 (1.5)	75
Group L <i>Streptococcus</i> CDC 1609-91	72 (2.0)	66	81 (1.0)	80
<i>S. canis</i> ATCC 43396 <sup>T</sup>	49 (ND)	ND	46 (ND)	ND
<i>S. canis</i> CA 146	48 (ND)	ND	46 (ND)	ND
<i>S. canis</i> CA 180	47 (ND)	ND	44 (ND)	ND
<i>S. equi</i> subsp. <i>equi</i> ATCC 33398 <sup>T</sup>	22 (ND)	ND	18 (ND)	ND
<i>S. equi</i> subsp. <i>zooepidemicus</i> ATCC 43079 <sup>T</sup>	25 (ND)	ND	21 (ND)	ND
<i>S. pyogenes</i> ATCC 12344 <sup>T</sup>	47 (ND)	ND	48 (ND)	ND
<i>S. iniae</i> CDC 2378-91	25 (ND)	ND	23 (ND)	ND

ND, Not determined.

\* % D, percentage divergence.

relatedness (Wayne *et al.*, 1987). Studies by Farrow & Collins (1984) and Kilpper-Bälz & Schleifer (1984), showed that the sub-groups of *S. dysgalactiae* belong to a single species on the basis of only high-level DNA relatedness to the type strain ATCC 43078. These hybridization studies were performed by the membrane filter method, and levels of divergence and DNA reassociation at a stringent temperature (70 °C) were not determined.

The results of our DNA-DNA reassociation experiments have shown that strains of all of the phenotypic sub-groups were genetically related at the species level. Two DNA relatedness sub-groups were present when the percentage of divergence within related sequences was considered. DNA relatedness sub-group 1 contained  $\alpha$ -haemolytic strains, and sub-group 2 contained the  $\beta$ -haemolytic strains. The results obtained by MEE correlated with results obtained by DNA-DNA hybridization. The  $\alpha$ -haemolytic group C strains that were clustered independently by MEE clustered in DNA relatedness sub-group 1. According to MEE data and DNA-DNA hybridization studies,  $\beta$ -haemolytic group L and group C (*S. equisimilis*) streptococci from animals were closely related to human  $\beta$ -haemolytic groups C and G strains. For strains of  $\beta$ -haemolytic group L streptococci, the levels of divergence in related sequences were slightly lower than observed in the other strains of this group. This sub-group belonged to cluster III as determined by MEE, and most of these strains were placed into a single subcluster (b). However, some of these strains remained in another subcluster (c) with human  $\beta$ -haemolytic group C and group G streptococci.

The sub-groups of *S. dysgalactiae* could also be differentiated on the basis of phenotypic characteristics. The  $\alpha$ -haemolytic group C strains were distinguished from human  $\beta$ -haemolytic *S. equisimilis* and also group G because they failed to produce  $\alpha$ -L-glutamate aminopeptidase,  $\beta$ -D-galactosidase and proteolytic activity on human fibrin. They were susceptible to bacitracin and most of them produced acid from sorbitol. Strains belonging to this sub-group differed from  $\beta$ -haemolytic group L strains on the basis of their inability to produce  $\alpha$ -L-glutamate aminopeptidase and  $\beta$ -D-galactosidase, and most strains fermented sorbitol. Human strains of  $\beta$ -haemolytic group C and G could be distinguished only on the basis of serological characteristics. These strains differed from group L strains in their ability to produce fibrinolysin, in their inability to produce acid from glycogen and in their resistance to bacitracin. In addition, some of the L strains hydrolysed hippurate. The *S. equisimilis* strains associated with animals could be distinguished from human *S. equisimilis* strains by glycogen fermentation and by failure to produce fibrinolysin. Overall, the results of biochemical reactions were comparable to the findings of Efstratiou *et al.* (1994) and Sippel *et al.* (1995). However, in other studies,  $\beta$ -D-galactosidase activity has been considered negative for human group C and G (Devriese *et al.*, 1986;

Efstratiou *et al.*, 1994) and for group L (Farrow & Collins, 1984) streptococci. In the present investigation, most of the strains were  $\beta$ -D-galactosidase-positive. Several authors have also found a high proportion of human group C, G (Slifkin & Gil, 1983) and group L streptococci (Sippel *et al.*, 1995) to be able to produce this enzyme. They also determined  $\beta$ -D-galactosidase activity with 4-methylumbelliferyl-conjugated  $\beta$ -D-galactoside as substrate.

Our data are in agreement with the division of *S. dysgalactiae* into two subspecies, but differ from the findings of Vandamme *et al.* (1996) in that  $\beta$ -haemolytic group L and group C (*S. equisimilis*) streptococci from animals were clustered together. The results of the present study also showed that the  $\alpha$ -haemolytic group C strains are distinct from the  $\beta$ -haemolytic group C, G and L strains. Therefore, we propose that they are placed in a distinct subspecies, *Streptococcus dysgalactiae* subsp. *dysgalactiae*. On the other hand, the  $\beta$ -haemolytic strains should be allocated to another subspecies, *S. dysgalactiae* subsp. *equisimilis*. Although there may be host-adapted ecovars within this subspecies, in the present report differences in DNA-DNA reassociation experiments correlated more with physiological variation than with host origin (ecovariation). This was exemplified by group C strains, and, particularly, by group L strains. Human isolates of group L were not distinguishable from animal isolates. Furthermore, group L strains could be discriminated from the *S. dysgalactiae* subsp. *dysgalactiae* strains but not from subsp. *equisimilis* strains. Although there is no practical reason to subdivide a species on the basis of DNA-DNA reassociation experiments, the two distinct DNA-DNA clusters of strains also correlated with physiological characteristics that can be recommended for the differentiation of strains, including the haemolytic activity. According to our proposal, the subspecies *dysgalactiae* contains only  $\alpha$ -haemolytic strains and the subspecies *equisimilis* contains all  $\beta$ -haemolytic *S. dysgalactiae* strains. Haemolytic activity is one of the most stable and classical phenotypic characteristics used to differentiate the streptococci and can be easily performed in diagnostic laboratories.

Phenotypic characteristics are also helpful in differentiating some of the ecovars. The group C *S. equisimilis* strains with the animal biotype can be distinguished from those with human biotype by biochemical tests. Strains of groups C and G streptococci isolated from humans appear to be very similar. Their homology is also suggested by similarities in some surface structures that are known to contribute to the virulence of the organism. Streptococcal IgG-binding proteins (Fc receptors) have been described for *S. dysgalactiae*. The type III Fc receptors from groups C and G streptococci (Björck & Kronvall, 1984; Sjöbrink *et al.*, 1991) have been shown to be similar. Jonsson *et al.* (1994) and Jonsson & Müller (1994) have recently cloned and sequenced two genes from *S. dysgalactiae* encoding Fc receptors with a new

molecular architecture. These proteins, called protein MIG and protein MAG, represent new variants of streptococcal IgG-binding proteins. Group L *Streptococcus* strains have Fc receptors, but information about their structure is scarce (Sippel & Lämmler, 1995). In addition, M-like proteins have been demonstrated in groups C, G (Jones & Fischetti, 1987) and L (Sippel *et al.*, 1995) but their occurrence in  $\alpha$ -haemolytic streptococci is unknown. The structural variations of Fc receptors and the presence of M-like proteins are consistent with our results and subspecies proposal.

Confusion regarding which strains to be included in *S. dysgalactiae* subsp. *equisimilis* strains is present in the literature. *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994) places the human group C streptococci in *S. equi* subsp. *equisimilis*. This nomenclature has also been adopted in a recent study (Turner *et al.*, 1997). The allocation of the human group C and G streptococci to the same subspecies will have epidemiological and clinical implications. In fact, most studies include only strains that exhibit group C or G antigen. Consequently, the frequency of *S. dysgalactiae* subsp. *equisimilis* as a cause of infections is undervalued.

#### Emendation of the description of *Streptococcus dysgalactiae* subsp. *dysgalactiae*

The characteristics of *S. dysgalactiae* subsp. *dysgalactiae* are the same as those described by Farrow & Collins (1984), with the following exceptions. The strains are  $\alpha$ -haemolytic and nonhaemolytic and react with Lancefield group C antigen. Streptokinase activity on human plasminogen does not occur (Vandamme *et al.*, 1996). Additional biochemical characteristics are shown in Table 2. These strains belong to DNA relatedness sub-group 1. This subspecies is commonly found associated with bovine mastitis and may also be isolated from bovine vagina.

#### Emendation of the description of *Streptococcus dysgalactiae* subsp. *equisimilis*

The characteristics of *S. dysgalactiae* subsp. *equisimilis* are the same as those described by Farrow & Collins (1984), with the following exceptions. All strains are  $\beta$ -haemolytic and may possess Lancefield group C, G or L antigen. Streptokinase activity occurs on human plasminogen (Vandamme *et al.*, 1996). Additional biochemical characteristics are shown in Table 2. There are several phenotypic categories within this subspecies which have been found in isolates from different animal ecovors. These ecovors are found in the normal human throat, vagina and skin and in other animal sites. They are also found in miscellaneous infections of humans and domestic animals. All the phenotypic categories belonging to this subspecies are included in DNA relatedness sub-group 2.

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